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Effects of Cadmium and Mercury on the Behavioral Responses and Development of *Eurypanopeus depressus* Larvae*

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Abstract

Larval stages of the estuarine mud crab *Eurypanopeus depressus* were exposed to either 10 ppb cadmium or 1.8 ppb mercury in a flow-through rearing system. Development time from the megalopa to juvenile crab was extended in the cadmium-exposed individuals. Cadmium elevated the swimming rates of the late zoeal stages, while mercury depressed swimming rates of the early stages. Increased mortality of Stage I zoeae was observed after 24 h exposure to cadmium; increased mortality was also noted for megalopa and early crab stages reared in cadmium.

Introduction

During recent years there has been considerable documentation of the deleterious effects of both cadmium and mercury on marine organisms (Gardner and Yevich, 1969, 1970; Eisler, 1972; Friberg and Vostal, 1972; Eisler and Gardner, 1973; Newman and MacLean, 1974; Schmidt-Nielson, 1974; Vernberg *et al.*, 1974; Waldichuk, 1974; Cunningham and Tripp, 1975; Nimmo and Bahner, 1976; Barthalamus, 1977). These metals have been introduced into estuarine and coastal waters through industrial discharge, fertilizer, fungicide and pesticide run-off, and through direct pesticide application (D'Itri, 1972; Friberg *et al.*, 1974; Huisingh and Huisingh, 1974). Earlier research concerning the effects of toxic metals and pesticides on marine organisms primarily emphasized short-term, lethal dose studies on adult organisms maintained under relatively constant environmental conditions. Recently, however, there has been extensive research demonstrating that low concentrations of a toxicant can markedly modify the physiological functioning of both larval and adult organisms, with larvae generally less tolerant than adult stages (Buchanan *et al.*, 1970; Epifanio, 1971; DeCoursey and Vernberg, 1972; Vernberg *et al.*, 1973; Nimmo and Bahner, 1976; Rosenberg and Costlow, 1976; Bresch and Arendt, 1977). Such sublethal effects involving alterations in normal growth and development, or in the ability to perceive and respond to the demands of the environment, will ultimately result in mortality and altered community dynamics.

One of the most critical concerns in evaluating laboratory toxicity studies is the relevance of such studies for animals in a complex natural environment. The testing of pollutant toxicity should theoretically be done under conditions which approximate the natural environment as closely as possible. Temperature, salinity, and photoperiod are the most easily regulated environmental parameters. Environmental water conditions, however, can only be approximated in the laboratory. The static experimental conditions widely used in larval crustacean research pose two important problems: (1) water stagnation; (2) maintenance of a constant concentration of pollutant due to animal uptake and the colligative properties of many toxicants. The objective of the present study was to assess the effects of low-level concentrations of cadmium and mercury on the survival, development and behavior of larvae using a flow-through system. The common estuarine brachyuran crab *Eurypanopeus depressus* was used.

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Materials and Methods

Gravid *Eurypanopeus depressus* females were collected from the intertidal oyster beds at the Belle W. Baruch Foundation near Georgetown, South Carolina, USA. The crabs were maintained in the laboratory in finger bowls containing 30‰ S double-filtered seawater at a temperature of 21° to 23°C. They were fed fish-fillet pieces twice weekly, and clean water was provided after feeding. The finger bowls were examined each morning for hatches. Hatches were considered vigorous when more than 90% of the larvae demonstrated a strong phototactic response. Vigorous hatches were pooled on Day 0 for experimental use.

For the experiments, approximately 300 zoeae were transferred to each control or experimental flow-through chamber, where they were maintained until metamorphosis. Each chamber had a 77 mm inner diameter (ID) x 134 mm insert, with a 200 µm Nytex mesh floor to facilitate removal of larvae. In addition, each chamber could accomodate a small insert (35 mm ID x 95 mm) containing zoeae for the developmental studies. Chambers were placed in an environmental chamber at 25°C and a 12 h light:12 h dark schedule. Newly-hatched *Artemia salina* were provided as food 2 to 3 times per week. The seawater flow rate of 13 ml min^{-1} was regulated by Buchler polystaltic pumps. A cadmium stock solution (2 ppm cadmium chloride) was dispensed into the inflow seawater lines by a Sage syringe pump to achieve a final concentration of 10 ppb. Fresh stock solutions of cadmium chloride were prepared monthly. A solution of 36 ppm mercuric chloride was introduced into the flow-through systems to maintain a final concentration of 1.8 ppb. Fresh stock solution (360 ppm) was prepared every 10 days. (For further details of the method see Vernberg *et al.*, 1977). Mortality was monitored by counting the larvae from the small inserts. Larvae were identified as Stages I, II, III, or IV zoeae, megalopa, or juvenile crabs (Costlow and Bookhout, 1961). Staging was delayed until Day 18 to reduce handling-induced mortality. Behavioral patterns were determined by measuring the larval swimming rates under uniform (random) lighting and directional lighting. Directional light was utilized to differentiate a random type of swimming from a phototactic response, normally exhibited by vigorous, newly-hatched zoeae. For the random swimming-rate measurements, zoeae were placed singly in a 30 mm finger bowl in 30‰ S filtered seawater. A camera-lucida drawing tube attached to a Wild Dissecting Microscope (7 x magnification) was utilized to track the swimming path for 30 sec in the uniform substage lighting. Four replicates were measured for each individual. For the directional swimming rate measurements, a point light source (Model 365 microscope light) was directed onto the test dish and the swimming path recorded for four 15-sec replicates. Travel rate was analyzed by measuring track distances. The patterns were measured using a K & E map distance measuring wheel; results were expressed as actual mean travel rates sec^{-1}.

One-sided non-parametric statistical analyses using the Wilcoxon rank sum test were utilized to determine level of significance. Differences at a level greater than $P = 0.05$ were not considered significant.

Results

The survival rates of 100 experimental and 100 control *Eurypanopeus depressus* zoeae were monitored for 100 days in the flow-through system. Relatively high mortality differences between control and experimental Stage I zoeae occurred during the first 24 h; subsequently, the mortality rates did not differ significantly until the onset of Crab I stage at 25 to 35 days. On Day 40, a total of 16 control and 6 treated crabs were alive (Fig. 1). The juvenile crabs were maintained until Day 100 to follow mortality through early crab stages. The control number remained constant on Days 40-100 at 16 individuals. On Day 44, the cadmium-treated juvenile crabs were placed in untreated seawater, where their numbers continued to decline until only 3 were alive at Day 100. Cadmium retarded the metamorposis from megalopa to crab: 8 days more were required to reach 100% crab (Fig. 2). Cadmium also increased the normal mortality occurring at the molt from megalopa to crab stages (Fig. 3). Mortality for the period from 100% megalopa to 100% crab was 43% for controls but 93% for cadmium-treated larvae.

No significant differences between swimming rates of Stages I or II control and cadmium-exposed zoeae were noted in a uniform-light field. Both Stage III and IV cadmium-treated zoeae exhibited a significant ($P = 0.005$) increase in random swimming rates compared to controls (Fig. 4a). No significant difference between the directional swimming rates of control and cadmium-exposed Stage I zoeae was noted (Fig. 4b). At Stage II, a significant ($P = 0.05$) elevation of the experimental rate was observed which

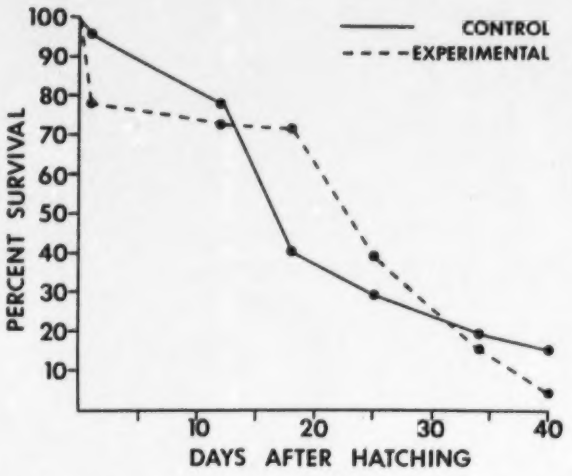

Fig. 1. *Eurypanopeus depressus*. Percent survival of control and cadmium-exposed larvae through Day 40, at which time both controls and experimentals had reached Crab I stage

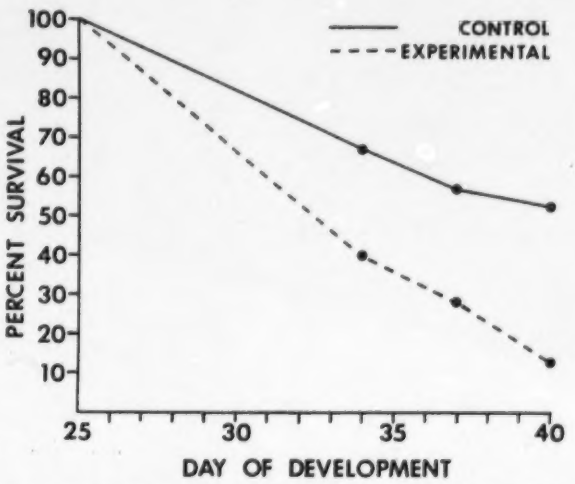

Fig. 3. *Eurypanopeus depressus*. Effects of cadmium on survival of megalopal and crab stages. Horizontal axis begins at Day 25, when 100% of the larvae attained megalopal stage

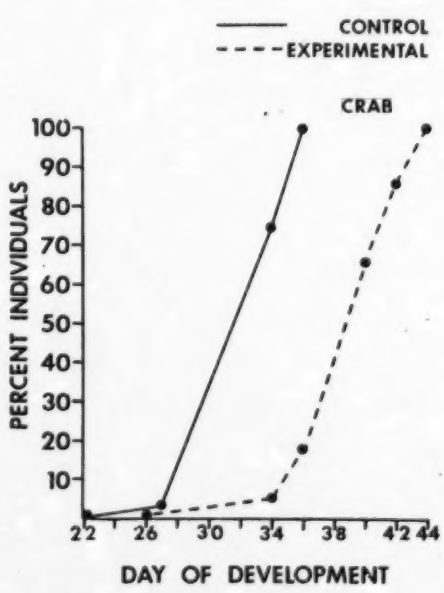

Fig. 2. *Eurypanopeus depressus*. Rate of development from megalops to crab of control and cadmium-exposed larvae from Day 22 through Day 44. Horizontal axis begins at Day 22, when control group reached 100% megalops. Cadmium-exposed group reached 100% megalops at Day 26. Data are graphed as percent individuals attaining a given stage against day of development

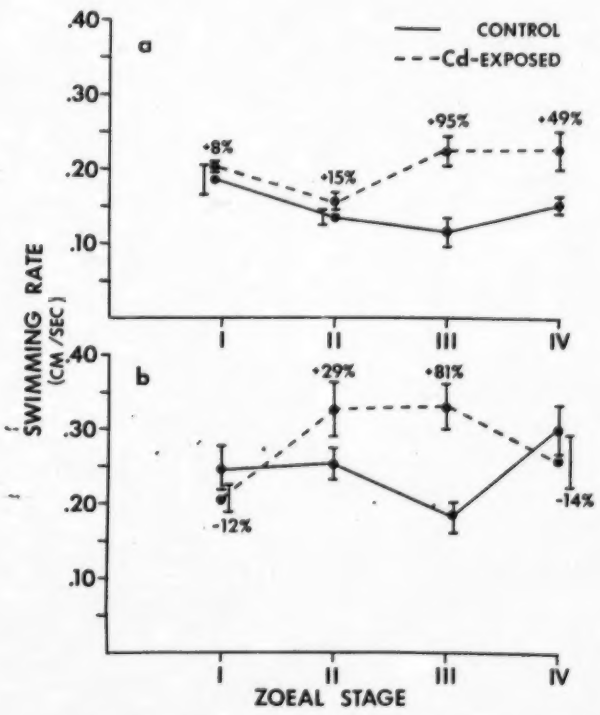

Fig. 4. *Eurypanopeus depressus*. (a) Mean random swimming rates of control and cadmium-exposed zoeae; (b) mean directional swimming rates of control and cadmium-exposed zoeae. Standard error is indicated by bars, and percent deviation from controls is numerically presented

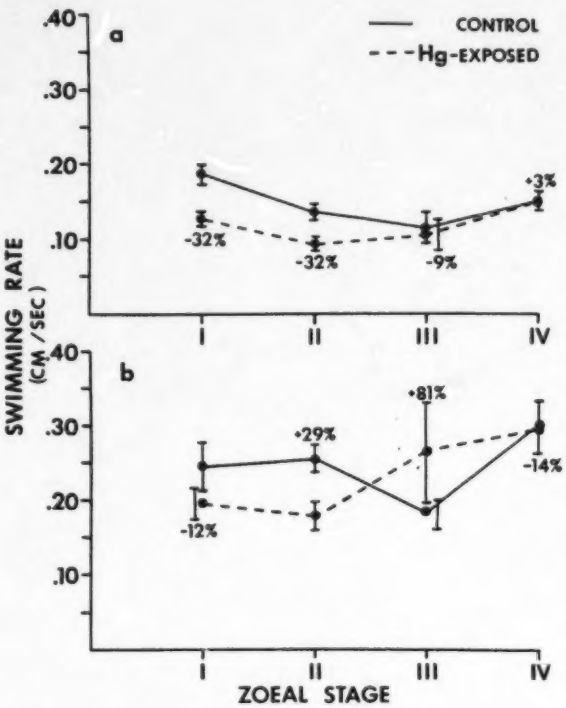

Fig. 5. *Eurypanopeus depressus*. (a) Mean random swimming rates of control and mercury-exposed zoeae; (b) mean directional swimming rates of control and mercury-exposed zoeae. Standard error is indicated by bars, and percent deviation from controls is numerically presented

persisted into Stage III (P = 0.005). The control and experimental swimming rates at Stage IV were not significantly different. An elevation in swimming rate is a general response of the zoeae to cadmium exposure. There appears to be stage-dependent variation between the random swimming response and the directional swimming response.

Mercury depressed the random swimming rates of Stage I (P = 0.005) and Stage II (P = 0.025). The Stage III and IV random swimming rates did not differ significantly from control values (Fig. 5a). The directional swimming rates were significantly reduced (P = 0.005) at Stage II (Fig. 5b). At Stages I, III and IV no significant differences were noted.

Discussion

Within recent years, there has been a growing recognition of the deleterious effects of low concentrations of metals on larval crustaceans (DeCoursey and Vernberg, 1972; Vernberg *et al.*, 1973; Shealy and Sandifer, 1975). Results of the present study indicate that chronic exposure of *Eurypanopeus depressus* larvae to sublethal cadmium concentrations results in changes in swimming rates, extended developmental time, and increased mortality, with differential toxicant sensitivity related to developmental stage. Similar results have been reported for the development of crustacean larvae following exposure to sublethal concentrations of pesticides (Buchanan *et al.*, 1970; Epifanio, 1971; Bookhout *et al.*, 1972; Roesijadi *et al.*, 1976; Bookhout and Monroe, 1977; Neff and Giam, 1977). Since chlorinated hydrocarbons are known to have an affinity for fatty tissues, Bookhout *et al.* (1972) speculated that the release of fat-stored pesticides into the bloodstream could be responsible for the observed mortalities during the megalopal stage of *Rhithropanopeus harrisii* and *Menippe mercenaria*. For metals, however, the mechanism of action is not known.

A comparison of the effects of mercury and cadmium on the swimming rates of larval *Eurypanopeus depressus* indicates that mercury tended to have a depressing effect, while cadmium acted as a stimulant. Not all stages were equally affected. A reduction in swimming rate was a general response of early zoeal stages to mercury exposure, while Stage III and Stage IV zoeae did not exhibit aberrant swimming rates. Cadmium effects were not significant until Stage III. Qualitative deviations from the normal swimming pattern were not observed. DeCoursey and Vernberg (1972) reported marked swimming abnormalities and a general reduction of swimming rate in mercury-exposed *Uca pugilator* zoeae. The later larval stages of that species exhibited a greater mercury sensitivity. They also observed depressed respiration rates, reduced survival, and a differential mercury sensitivity between stages.

In conclusion, this study has shown that sublethal concentrations of mercury and cadmium alter normal survival rates, development, and swimming behavior of *Eurypanopeus depressus* larvae. The modification of any of these parameters ultimately results in reduced numbers, and potentially affects community dynamics. Implications which follow from this research and the other larval studies for toxicology studies are clear. Sublethal toxicant studies must supplement lethal dose studies and representatives of each developmental stage should be used in pollution bioassay research. Finally, the question of synergism of stresses in the natural estuarine environment cannot be ignored. The suboptimal conditions which naturally occur in the estuarine environment can only amplify the threats posed by continued pollution.

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